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# NATURE AND ORIGIN OF THE BI-NUCLEATED CELLS IN SOME BASIDIOMYCETES.

SUSIE P. NICHOLS.

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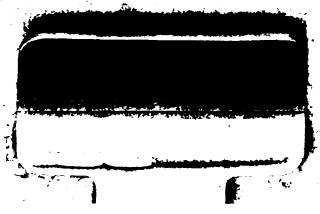

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# THE NATURE AND ORIGIN OF THE BINUCLEATED CELLS IN SOME BASIDIOMYCETES

BY

SUSIE PERCIVAL NICHOLS

A THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
UNIVERSITY OF WISCONSIN
1904

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# THE NATURE AND ORIGIN OF THE BINUCLEATED CELLS IN SOME BASIDIOMYCETES.

SUSIE PERCIVAL NICHOLS.

## INTRODUCTION.

Rees (20) was among the first to attempt a careful study of the mycelium of the *Basidiomycetes* with reference to the question of the origin of the carpophore. By making artificial cultures of *Coprinus stercorarius* in dung decoction on slides he was able to observe the formation of erect short hyphae on which he believed sexual cells, spermatia and carpogonia were borne. He also believed that he found a spermatium fused with a carpogonium. After fertilization, branches arise from the base of the carpogonium which developed into the carpophore.

Van Tieghem (25) also germinated the spores of *Coprinus stercorarius* and *radiatus* in dung decoction and studied the development of the carpophore. In his first paper he agreed with Rees. He found the swollen end cells on the lateral branches of the mycelium. These "carpogones" usually terminated in a papilla with which the spermatia fused. The carpogone then divided into three cells, the two lower developing a system of lateral branches which curve around and enclose the terminal cell. Their further development in slide cultures was prevented by lack of nutriment. But by observations made on larger cultures they were seen to be the beginnings of carpophores. Later Van Tieghem reversed this opinion.

Brefeld (3, 4) grew mycelium of *Coprinus stercorarius* from single spores in dung decoction and figures a series of stages in

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the development of the young carpophore. From the older portion of the mycelium a perpendicular hypha very rich in protoplasm arises. This hypha branches profusely forming a dense snarl from the center of which a bundle of parallel hyphae develop forming the first indication of the stipe. Lateral branches are formed increasing the size of the mass and at the same time the stipe grows rapidly in length. The pileus and gills are differentiated very early in the development of the fruit body. Brefeld found no evidence of the existence of sexual organs at the formation of the carpophore.

With the study of the nuclear phenomena new stand-points arose. The work of Rosen (21), Rosenvinge (22), Wager (26, 27, 28), and Dangeard (5) has established the fact that the cells of the carpophore are frequently multinucleated while the basidia are at first binucleated. In typical basidia the two nuclei fuse and the fusion nucleus divides into the four spore nuclei.

Maire (15) found that the cells of the young carpophore are binucleated and that the cells of the hymenial layer never have more than two nuclei, but that the cells of the stipe and pileus may become multinucleated through the amitotic fragmentation of the two nuclei originally present. The young basidium when it is formed from the hymenial cells receives two and only two nuclei which unite to form the large fusion nucleus of the basidium. He further states that the nuclei in the series of binucleated cells in the young carpophore divide by conjugate divisions so that the two nuclei which fuse in the basidium are of widely different origin. But his evidence is not conclusive on this point.

Maire describes the division of the nucleus in the basidium in detail. The nuclear membrane disappears and the spindle appears at about the same time. The chromatin filaments break up into irregular granules or protochromosomes which are placed on the spindle without any definite order. At the end of the prophase these protochromosomes unite into two definite chromosomes. That the formation of only two chromosomes is not universal among the Basidiomycetes as Maire assumes has been shown by Wager, Ruhland and others. Maire states further

that the chromosomes after a longitudinal splitting are pulled apart at the center and move to the poles. The second division is similar to the first. The four centrosomes remain at the summit of the basidium while the nuclei move to the center or base of the basidium. Soon a sterigma is formed above each centrosome, and fibres appear extending from the centrosome to the nuclei which now move to the summit, probably through the influence of these fibres.

Two notes of Maire's (16 & 17) in the Comptes Rendus report that the last two or three cells of the ascogenous hyphae of *Pustularia vesiculosa, Galactinia succosa* and *Acetabula acetabulum* are binucleated. The ascus like the basidium is the last of a series of binucleated cells. In order that such a comparison should have any value we must know how the ascogenous hyphae originate.

In *Hypochnus* Harper (12) was able to trace a series of binucleated cells from the hymenium to the mycelium in the substratum. The mycelium did not form dense wefts or strands but spread through the decaying wood where it could be readily studied. The cells were regularly binucleated. The stages of nuclear fusion and division were similar to those described by Wager. At the equatorial plate stage the chromosomes were distinct and at least six or eight in number.

The origin of the binucleated cells was not determined by these observers. Maire states that the two nuclei in the spore of *Coprinus radiatus* pass into the germ tube and a cross wall may or may not be formed between them. The mycelium is then of two types, the one apocytic, the other cellular. The cells of the latter are uninucleated. He did not observe the transition from these stages to the binucleated condition found in the young carpophore.

In a preliminary notice Blackman (2) has given a brief account of the life history of two of the *Uredineae*. He finds that the spermatia do not have the structure of conidia but of male cells; a thin wall, no reserve material, a very large nucleus with no nucleolus and cytoplasm greatly reduced in amount. He also studied in detail the development of the aecidium of *Phragmidium violaceum*. The aecidium arises as a layer of uninu-

cleated cells just beneath the epidermis of the leaf. Each of these cells divides into a sterile cell above and a fertile cell below. The fertile cell becomes binucleated not by the division of its original single nucleus but by the migration through the wall of the nucleus of a neighboring vegetative cell of the mycelium. He says, "In the presence of the spermatia with their special cytological characters, etc., the only view that seems capable of explaining the facts is that the fertile cell was formerly fertilized by the spermatia, but that now the process has become reduced, fertilization by means of spermatia having been replaced by the more certain method of fertilization by the nucleus of a neighboring vegetative cell." In view of these facts he holds it to be evident "that the Uredineae present an alternation of generations which is as sharply marked as that of the higher plants."

Since the young carpophores invariably have binucleated cells these must originate either at the first formation of the carpophore or sometime during the growth of the mycelium. The latter hypothesis is suggested by Harper's observations on *Hypochnus*. In order to obtain some evidence on this point the study of the nuclei in the mycelium of some of the *Agarics* was begun at the suggestion of Professor R. A. Harper under whose direction the work was carried on.

## METHODS.

Spores were collected from a large number of *Agarics* in the following manner. Zinc racks were washed in alcohol and passed through a flame and then placed in plates which had been washed in 95 per cent. alcohol. The racks were covered with bell jars which were also washed in 95 per cent alcohol. Two racks were placed side by side under each jar. Slides were washed in alcohol and passed through a flame and then placed on the lower bars of the racks, each rack holding four. The pileus from a mature fruit body was carefully removed from the stipe and placed on the upper bars of the rack. When the basidia discharge their spores they fall on the slides below thus lessening

the danger of infection from the gills. After the spores were discharged the pileus was carefully removed and the bell jar replaced the slides remaining on the racks until needed. Spores preserved in this manner remained pure for a year.

A decoction of string beans, (about 392 grams to a liter of water) proved to be the best nutrient although a decoction of *Coprinus* and of dung was used for some forms. For the early stages in the development of the mycelium small cultures were made in dishes holding 10 c. c. of the nutrient solutions. Large quantities of spores were sown and at the end of 12, 24, and 48 hours, the nutrient solution was removed by a pipette until only 2 c. c. remained, the dish was then filled with fixing solution which would thus be reduced about one-fifth in strength. After fixing 24 hours the spores were stippled on the slide by the method described by Harper (11) in his paper on the nuclear phenomena in the smuts.

Spores were also sown in thin films of agar-agar on sterilized slides. When the mycelium had attained the desired growth the entire slide was immersed in fixing solution. If the film loosened from the slide it was easily fastened again by a film of albumen.

To obtain mycelia, cultures were made similar to those described by Falk (8). Rye bread cut in slices two or three inches thick were moistened in bean decoction and fitted into battery jars five inches deep by four wide. For covers petri dishes four and a half inches in diameter were used. A thin layer of cotton was placed between the cover and the dish to allow free circulation of air. Agar-agar plates were also used.

The spores germinate in from six to eight hours and at the end of two or three days form a growth of mycelium which appears as a white mat about a quarter of inch in diameter on the surface of the bread. The mat increases in size rapidly until it is two or three inches in diameter. At the same time there appear all over the culture small white dot-like masses of mycelium. These are new growths from oidia scattered from the first mycelium. Falk has also described and illustrated such oidial colonies. These small secondary growths were removed

whole with about a quarter of an inch of the substratum. Larger mycelia were cut into small pieces for fixation. In order to force the fluid through the thick felt which the mycelium forms the material was placed in a small bottle and well covered with the fixing fluid. The bottle was then fitted into the end of a rubber tube which was connected with the air pump. The air was pumped out from the closely matted hyphae, after which the fixing fluid was renewed.

The material was fixed in Flemming's solutions both the stronger and the weaker. Merkel's and Herman's solutions were also used. The best results were obtained from Flemming's weaker solution. Both Flemming's triple stain and Heidenhain's iron haematoxylin gave satisfactory results.

### *Hypholoma perplexum*, Pk.

The spores of *Hypholoma perplexum* were collected in great abundance. The carpophores appeared in great profusion on decaying stumps and logs of oak throughout September and October. The spores were collected and stored after the manner already described. A large per cent of the spores obtained from mature pilei germinated in a shorter time than those obtained from younger ones. The spores were sown in the hanging drop agar cultures made with bean decoction. The cultures were kept in a dark box at a temperature of 20° c. As all the spores do not germinate at the same time but vary from four to forty-eight hours in the time of the appearance of the germ tube a large number of stages are obtained from the same slide.

The mature spores were studied in the drop cultures and also in the cross sections of the gills where they were still in connection with the sterigmata.

The mature spore of *Hypholoma perplexum* has a dark brown opaque wall which before germinating swells to two or three times its original thickness becoming much lighter in color and transparent. At this time it is easy to distinguish two nuclei lying close together near the center of the spore. They show the usual structure of the resting nucleus and are small spherical bodies with a well defined membrane, a large distinct nucleole

and a fine granular chromatin which fills the remaining space (Fig. 1). The chromatin stains a light blue with the triple stain while the nucleolus stains with safranin. Previous to germination there is no indication of a germ pore but the germ tube always appears at the same place on the spore. On germinating a tube in the form of a large round bulb of protoplasm is protruded from the end opposite the point of original attachment to the sterigma. The germ tube increases rapidly in size retaining its spherical form as the cytoplasm passes into it from the spore. One of the nuclei passes from the spore into the tube as soon as there is room (Fig. 2 & 4) the other may pass out at once and it will then be seen near the first (Fig. 3) or it may remain in the spore for some time. Strands of cytoplasm caused by streaming from the spore into the tube are clearly shown in many of the preparations. As the nucleus which has remained in the spore is carried along by the streams of cytoplasm it becomes drawn out into a blunt point on the side towards the germ tube. (Fig. 2). As the nucleus approaches the narrow passage from the spore into the germ tube it is very nearly cone shaped. The vacuoles are very small at this stage with the exception of a single large vacuole which forms in the spore back of the second nucleus.

The tube begins to lengthen immediately in the direction of the long axis of the spore. The bulb has not formed a firm wall at this time and as the tube lengthens it becomes drawn out in the general direction of growth until its diameter is only slightly greater than that of the spore. The two nuclei do not remain together, one moving towards the apex of the tube while the other remains near the base. One of the preparations at this stage showed late division figures (Fig. 5). The chromosomes have already passed to the poles so that it is impossible to determine their number. The spindle fibres still connect the two masses. The two nuclei lie at some distance from each other. One spindle which is near the tip is nearly parallel with the walls of the germ tube. The spindle of the other, near the spore, is placed obliquely. Near one of the nuclei are two small deep staining bodies which may be remnants of the old nucleoles. (Fig. 5a). In an older germ tube the next division was ob-

served. In this hypha three nuclei were in the resting condition. One is somewhat separated from the others towards the tip of the hypha and is a densely staining homogeneous body. Two other nuclei side by side near the center are elongated with the nucleole at one end. The fourth nucleus is dividing and is in the equatorial plate stage. The separate chromosomes could not be distinguished. The spindle fibres show an even distribution at the center and are collected into a definite pole. The presence of a central body could not be distinguished definitely (Fig. 6).

As the germ tube elongates the cytoplasm forms a much thinner layer at the periphery of the cell and a number of large vacuoles appear. The number and size of the vacuoles increase until they are only separated by fine lamellae of granular cytoplasm. The nuclei multiply rapidly and are frequently in pairs for some distance but this arrangement is not at all uniform. The nuclei may be separated from each other by long distances or they may lie in groups of three or four. Cross partitions are not formed in the young germ tube. It frequently branches two or three times before the first cross wall is formed. The cells which are then formed are very variable in length. The number of nuclei which they contain is from one to four (Fig. 7). The mycelium does not consist in these earlier stages of regularly uninucleated or binucleated cells. The mycelium grows rapidly and branches freely and the branches interlace forming a loose net-work. The branches do not show any regularity in their origin. A few are formed near a cross wall but the majority bud out somewhere near the center of the cell. The majority do not show a cross partition at their base. The first cross wall appears at varying distances up the branch. Usually a nucleus is found at the base of each newly forming branch but this rule is not constant for in a few cases a fairly long branch was found without any nucleus near. The lateral branches are narrower than the hyphae from which they arise. The first few cells of the branch contain as a rule fewer nuclei than the cells in the main hypha but the cells nearer the tip may have a large number. The number of nuclei at this stage varies from one to eight or nine; the same branch frequently

showing both extremes in number. The nuclei are slightly smaller than those in the young germ tube but show the same general structure, a definite membrane and a nucleole which appears separated from the finely granular chromatin by a small clear space. In some of the preparations the nuclei were drawn out into long rather slender bodies with larger and very irregular chromatin granules loosely scattered throughout their length.

Special lateral branches may also be formed which have regularly uninucleated cells. These branches vary considerably in length and general shape. Some are long and nearly straight, or only slightly coiled, with long slender cells. The nucleus lies near the center of the cell and has the structure described above. Towards the ends of these branches very short cells are formed, only about twice as long as broad. These cells separate readily thus forming oidia. The character of the cytoplasm in these oidial cells is the same as that of the branch from which they originate. It is sometimes dense with very small vacuoles similar to that found in the spore. In other cases it has much larger vacuoles and the cytoplasmic granules as much larger and more irregular. In the majority of cases the nucleus is relatively large occupying nearly the entire diameter of the cell. In some instances the nuclear membrane and nucleole could not be distinguished and the chromatin was collected in two or four deeply staining masses usually oblong in shape. These nuclei may have been in some stage of division.

In one instance a slide culture that was placed in a very warm moist chamber (22° c.) showed a pronounced modification in the usual habit of growth. In many cases the germ tube was divided at once into uninucleated cells that bore numerous short series of oidia. The branches were coiled about the tube making it impossible to determine their number or structure. In other cases the hyphae were much longer and very slender with two or three branches on whose ends were large tangled masses of oidia. The oidia were rather slender and long but showed the usual structure.

Carpophores were not formed in any of the cultures but my studies on *Hypholoma perplexum* were continued on material

which had developed spontaneously in nature. During the winter specimens of *Hypholoma perplexum* appeared on an oak log in the green house. The rotten pieces of wood were broken away exposing an expanded sheet-like rhizomorph or perhaps sclerotium, with some young fruit-bodies just forming. A sharp distinction between rhizomorphs and sclerotia probably cannot be made. Between the round tuber-like sclerotium of *Coprinus ephemerus* and the long branched mycelial strands of *Armillaria mellea* are many intermediate forms, among which are the sheet-like mycelial masses of *Hypholoma perplexum.* These have an outer layer of hard brown cells and a central mass of thin walled hyphae bearing, perhaps, a closer resemblance to the structure of the sclerotium than to that of rhizomorphs. But the sheet-like masses do not have any definite shape and, as far as this material showed, may be unlimited in their growth—characteristics not usually associated with a sclerotium. The central portion of this hyphal mass is formed of parallel hyphae which do not show any protoplasmic contents. Their walls have become gelatinous and in the triple stain become light blue. Near the surface on each side there is a layer of thin walled hyphae with irregular cells. The cells are very closely packed together forming a pseudoparenchyma. On the outer surface there is a layer formed by small closely packed cells which are filled with a hard brown substance. When a carpophore is to be formed some of the thin walled hyphae force their way through the hard outer layer where they unite to form the carpophore. Occasionally the carpophores are formed singly but they are usually in clusters.

Closer examination shows that the thin walled hyphae just beneath the surface of hardened cells are formed of short irregular cells that contain very little granular cytoplasm. They are regularly binucleated. The nuclei are large, with a distinct nuclear membrane and a small nucleole. The chromatin does not appear as granular as in the majority of nuclei but takes a uniform light blue stain. The two nuclei are usually pressed close together near the center of the cell. In forming a carpophore a number of the thin walled hyphae force their way through the outer crust, branch profusely at the surface and spread out

slightly to form abroad base for the carpophore. The branches are short and branched in turn to form a knot of hyphae which is somewhat similar to the tangled mass formed at the base of the carpophore of *Coprinus.* But the cells are shorter and straighter so that the hyphae are not so matted and difficult to trace. Above the tangled hyphae at the base, the branches gradually assume a more vertical direction. They are still interlaced like the meshes of a net which has been pulled out lengthwise. Near the top of the button the number of branches increase and instead of continuing in a vertical direction they spread out radially. This is the first indication of a pileus. The outer branches of the central system of hyphae are not so closely interlaced but form a loose open network covering the pileus and stipe still further to the exterior. The surface of the button is formed by a layer of nearly straight hyphae. They arise as lateral branches near the base of the button and covering the entire surface, disappear among the hyphae at the apex of the pileus where the loose network of branches is thicker than at the sides. These hyphae are formed of long slender cells with very little protoplasmic contents. The shape of the cells and the direction of growth of the hyphae differentiate this outer layer very sharply from the tissues in the interior of the button. All of the cells are binucleated. The nuclei are large and have a nuclear membrane, a small nucleole and very finely granular chromatin.

At this time the young carpophore is an oval body and as indicated its tissues can be separated roughly into three distinct portions. The central portion formed of closely interlacing hyphae, which may be still further divided into the base of closely packed hyphae, the more vertical intervening hyphae from which the main portion of the stipe is formed, and the rather loosely spreading mass at the top from which the pileus develops. Second and outside of this central portion there is a poorly defined layer of loosely tangled hyphae which will untimately form the veil above and the outer covering of the stipe below. This layer is not sharpely separated from the central portion and is formed by lateral branches from that region. The third and outer portion is a layer of parallel hyphae

which form the external covering. This covering is doubtless a simple type of volva.

In a carpophore which is a little further developed the cells above the basal tangle have enlarged and elongated. By the increase in size of the cells the hyphae are pressed close together and with the elongation of the stipe are forced into very nearly vertical series. At the same time the cells in the pileus increase in size but remain more or less intertwined. There is no differentiation of hyphae for the formation of gills as yet. As the stipe elongates the outer covering of parallel hyphae is irregularly broken in several places, and now appears as small particles or scales which soon disappear. The protoplasm in the cells, which are rapidly increasing in length, forms a much thinner peripheral layer, the central vacuole having increased greatly in size. A longitudinal section through a young carpophore just before the breaking of the veil shows the gills already formed. Sections which pass through the center of a gill show the structure very clearly. The hyphae that spread out to form the pileus are very irregularly twisted about each other. Near the lower side of the cap there is a narrower layer of much straighter hyphae which extends from the stipe to the margin of the pileus. From the lower half of the pileus but especially from this layer of straighter hyphae, branches are formed that grow downwards in parallel series forming the trama. The hyphae of the trama branch freely, the branches forming a layer near the surface of the gills, the subhymenium. The hymenium is formed of closely packed basidia which are terminal cells of the hyphae in the subhymenial layer. Fig. 24 is from a section showing the connection of a basidium with the subhymenial hyphae. The large basidium has two nuclei lying near together at the center of the cell which is separated from the stalk cell by a cross wall. A second basidium is just forming from the basal cell but does not as yet contain any nuclei.

The hyphae at the margin of the pileus are continuous with the outer covering of the hyphae on the stipe and form the veil. The original external covering of parallel hyphae has disappeared with the exception of a few small fragments near the base of the stipe. All of the cells are regularly binucleated.

When the basidia are formed there is an uninterrupted series of binucleated cells from the binucleated cells of the sclerotium through the stipe, pileus, trama and subhymenium to the basidia. Later the cells of the stipe and pileus contain from six to eight nuclei.

The stages of nuclear fusion and subsequent division in the basidium have been fully described by Wager, Harper and others. In nearly all the essential points *Hypholoma perplexum* agrees with the forms studied by these authors. The nuclei are in the spirem stage at the time of fusion. The fusion nucleus moves to the summit of the basidium when it divides. The process of the formation of the spindle was not studied. The spindle is always at a right angle to the long axis of the basidia. The chromosomes in the equatorial plate show a tendency to aggregate into a number of larger masses probably as a result of fixation. In every case there are eight or more. Certainly there are many more than two as claimed by Maire.

The second division follows the first very closely. The chromosomes in this case are not scattered on the spindle but definitely arranged in an equatorial plate. The four nuclei move to the center of the basidium. At this time or a little later fibres extending from the sterigmata to the nuclei at the center were observed in a large number of basidia, but their origin was not worked out. As to these fibres Maire says "Après la formation des noyaux-fils dèfinitifs la baside, ceux-si se massent le plus souvent à la basè ou au milieu de la cellule, tandis que les centrosomes restent au sommet si les mitoses étaient apicales, s'y rendent dans le cas contraire. En face de chaque centrosome apparaît l'èbouche se produit une diffèrenciation kinoplasmique qui se propage jusque' aux noyaux at mème quelquefois plus loin vers la base de la cellule, orientant tant le cytoplasma de la baside par rapport aux sterigmates."

Each spore receives a single nucleus which immediately divides. In a number of cases the spindle is at right angles to the long axis of the spore. The chromosomes both at the equatorial plate and where they are drawn back to the poles appear to be four or more.

*Coprinus.*

The dung inhabiting *Coprini* are so easily obtained from dung cultures that the spores were not collected and stored as for the other species studied. The spores were transferred by means of a sterilized needle directly from the gills to the culture medium. For the study of the germinating spore and the early mycelium the agar hanging drop cultures are the most satisfactory. But for the older mycelium and for the formation of the carpophore agar plates in Petri dishes give the best results.

The spores of *Coprinus ephemerus* (?) germinate in from four to forty-eight hours. In the majority of cases of spores obtained from mature carpophores germination occurred in six hours. The spore wall does not swell and become transparent in germination as in *Hypholoma perplexum* and it is impossible to observe the position of the nuclei in the spore. At one end of the spore there is a thinner place in the wall—the germ pore. The germ tube appears as a small globular protrusion. One of the nuclei passes out from the spore at once into the germ tube. The nuclei divide very rapidly so that the young germ tube soon contains from three to six nuclei. At this time a nucleus can frequently be seen in the spore either near its center or just passing through the pore into the germ tube. At the end of the spore opposite the germ tube there is a dark hemispherical body, which perhaps marks the position of a second spore that has been closed by a cellulose thickening (Figs. 8 & 9).

The tube grows very rapidly forming a main hypha which is a direct extension from the spore and also a lateral branch which is nearly at right angles to it. This is well illustrated in Fig. 10 which is drawn from a germinating spore at the end of sixteen hours. The main hypha is slightly constricted at irregular intervals and in nearly every instance it is possible to determine the presence of a cross wall at the constrictions. The number and size of the nuclei vary in the different cells. The first and fourth cells counting from the spore have a very large nucleus which is more than twice the size of the nuclei in the other cells. The nucleole is very large and the chromatin is very much denser than in the nuclei usually found in this species. The other cells

of the main hypha have from one to three small nuclei. The lateral branch does not show any of the constrictions found in the main hypha. It is very difficult to locate the cross walls since when newly formed they are extremely delicate and easily confused with a strand of cytoplasm. In one of the cells there are fourteen very small nuclei many of which are only slightly larger than the nucleole of the large nuclei in the main hypha. They have a nuclear membrane, a minute nucleole and a few scattered granules of chromatin. The other cells of the branch have one or two large nuclei.

At the end of twenty-four hours a well developed mycelium is formed. Fig. 11 is drawn from a mycelium that was formed in a hanging drop culture in twenty-four hours. In addition to the main hypha and the lateral branch mentioned in the previous description a second smaller branch is formed close to the spore. These hyphae branch repeatedly, without any regularity. The original hyphae are larger and straighter than any of the lateral branches and for convenience in distinguishing them I shall call them the primary hyphae. In them the cells are very unequal in length but the majority are from seven to eleven times as long as they are broad. The number of nuclei varies also. The cell next to the spore contains a very large nucleus in the center and a small one lying at one side near the junction of one of the branches. The next few (five or six) cells of each of the primary hyphae have three or four nuclei which are slightly smaller than the one found in the first cell. The nuclei are usually near together at the center of the cell but in one or two instances one of them is slightly separated from the others. The next cells for some distance (eight or nine cells) have two nuclei which may be close together or separated. In the remainder of the hyphae the nuclei are separated at some distance from each other but in many cases it is impossible to distinguish a cross wall between them.

The lateral branches are much smaller and more irregular than the primary hyphae and the cells are shorter, three or five times as long as they are wide, and usually contain a single nucleus although cells containing four or five are frequently found. In a few instances where the branches extend to the

edge of the agar the terminal cells become smaller and contain a large number of nuclei closely packed together.

Arising from some of the cells near the base of the primary hyphae are short thick conidiophores. Their cells are nearly as broad as they are long. The end cells bear one or two very short branches which produce clusters of very slender hyphae formed of cells two or three times as long as broad. The cells separate readily into rod shaped oidia (Fig. 12). These hyphae do not appear above the surface of the substratum.

Near the center of the oidium there is a small well defined nucleus in which the nucleole and granular chromatin can be distinguished. The remainder of the cell is filled with finely vacuolated cytoplasm. This is in marked contrast with the form found by Maire on *Coprinus radiatus*. He says "Chaque oïdie renferme un seul noyau rèduit d'ordinaire à une petite masse homogène chromatique et un grande vacuole." On account of the number of oidia I was unable to see the nuclei in the cells of the supporting branch.

Occasionally the cells of the main hypha are binucleated. In this case the first few cells near the spore have from one to five nuclei. The cells further from the spore are regularly binucleated. At the end of five or six days branches appear on some of the older cells near the spore that are much larger than any previously formed and are frequently club-shaped. The cells of these branches are binucleated, the nuclei lying near together, sometimes side by side, near the center of the cell. The branches with binucleated cells may be formed from any cell of the mycelium near the spore. Fig. 13 shows one of the branches with binucleated cells formed on a hypha with uninucleated cells. The branch is formed very near the center of the cell instead of at one end as is usual with the mycelial branches. In my opinion the newly formed branch probably received a single nucleus which immediately divided. Fig. 14 is drawn from a hypha with multinucleated cells showing one of the larger branches just forming. Two nuclei of the multinucleated cell are just at the base of the newly forming branch.

Fig. 15 shows a group of these branches closely crowded together—the first indication of a fruit body. The hyphae have

many branches spreading outwards. In the center the hyphae are so densely packed that it is impossible to tell whether the branches originate from a single hypha or from several. As a number of hyphae from different directions in the substratum lie near together at this point it is probable that they arise from several.

The cells are regularly binucleated. The cytoplasmic strands are very delicate and form a loose network at the periphery of the cell. Very small convex plates are present at the cross walls marking the regions of protoplasmic connection between the cells. This method of formation of the carpophore was repeatedly observed in the hanging drop cultures. It is probably an adaptation to the culture conditions, especially the small supply of nutrient. In larger cultures of agar-agar in Petri dishes I find quite another method for the formation of the carpophore.

In an agar culture in a Petri dish the main hyphae with their system of branches can frequently be traced for one or two inches. Fig. 16 was made from the end of one of these systems. Squares of the agar a little smaller than a 7/8 inch cover glass can be fixed in Flemming's weaker solution and then fastened to a slide with a film of albumin. Preparations are best stained with iron haematoxylin. The stain washes from the agar in the iron solution leaving the mycelial hyphae sharply defined so that they can easily be studied.

The main hyphae are formed of very long cells which have from five to eleven nuclei that are smaller than in *Hypholoma,* but show the same general structure, they are elliptical with a nucleole at one end, finely granular chromatin, and a small body which is usually opposite the nucleole. This small body is flattened against the nuclear membrane and takes a very deep blue stain. The great regularity of its occurrence and its staining qualities have led me to consider this as the same structure as the central body which Harper described in the ascospores of *Erysiphe.* The primary branches which arise from the main hyphae show great regularity of arrangement. They are formed in pairs at one end of every cell or every other cell. They are never exactly opposite, one always appearing slightly below the other. These branches are approximately of the same size as

the main hypha and their base is enlarged in the same fashion as is the base of the germ tube. The young branch receives a single nucleus from the parent cell. A cross wall is then formed separating the branch from the main hypha while it is still very small. The nucleus divides very rapidly so that the young branch soon contains two or three nuclei. These continue to multiply until the mature cells contain as many or nearly as many nuclei as the cells of the main hyphae. The clamp connections between adjacent cells are quite abundant although they are not formed at every cross wall. They are cut off from one of the cells by a cross wall which always shows the deeply staining convex plates originally described by Hoffman (13) and since by Strasburger (24) and other authors. In every instance they show the same cytoplasmic structure as the cells which they connect. I have found one or two cases in *Coprinus* in which a branch has arisen from one of these clamp connections.

The secondary branches which arise from the primary hyphae do not show the same regularity in their formation. Only one is formed from a cell. They are very long and slender and run in every direction through the substratum. They anastomose with other branches frequently thus increasing the complexity of the hyphal system. The nuclei are separated by long distances, they are elliptical with a slightly larger nucleole than is found in the nuclei of the main hyphae. The cross walls are difficult to locate when, as frequently happens, the convex plates at the cross walls are absent.

The mycelium does not appear at the surface of the culture until a fruit body is to be formed. At this stage the character of the branching of the mycelium changes. The cells are shorter and branch three or four times, each successive cell branching in the same way. The cells are also curved backward towards the main hypha, thus a tangled mass is formed (Fig. 17). Other hyphae from various directions pass into this snarl and branch in the same way increasing its size and complexity. Fig. 17a is from one of these tangled masses and shows the Y shaped cells which are frequently found near the center. The cell walls are thick and tinged with yellow. The clamp cells are found at every cross wall connecting the adjacent cells.

All of the cells contain two nuclei which may lie close together but are more frequently separated by the entire length of the cell. In the Y shaped cells I have generally found one nucleus in each of the arms.

In my cultures the hyphal masses developed into carpophores only when they were formed near the surface or some break in the agar. A large number of small tangled masses were formed throughout the cultures but they did not develop into carpophores. Fig. 18 is from a section through a young carpophore which is developing from one of these tangles. The mass of hyphae is much larger than in the earlier stages but it shows the same structure. A large number of hyphae are found converging from different directions in the substratum to form by the interlacing of their branches near the surface of the agar this large tangled mass. The hyphae are so closely packed and so crooked that it is impossible to trace the cells their entire length but as far as I can determine they are in all cases binucleated. The hyphae outside of the tangled mass that are growing towards it and whose branches form the tangle invariably have binucleated cells. In the younger tangles the cells are binucleated and in this large hyphal mass the nuclei are usually found in pairs.

A more complete series of young carpophores of *Coprinus ephemerus* were obtained from dung cultures in the green house. By fixing the young carpophores when they first appear as small white dots on the substratum the main stages in the early development of the fruit body are very easily obtained. They are very similar to the corresponding stages of *Coprinus stercorarius* as described by Brefeld.

The very young carpophore is an oval body formed by a central mass of closely tangled hyphae and an outer covering of very loosely interlaced hyphae. The hyphae near the center are copiously branched. The branches branch in turn and gradually force their way upward and outward, the density of the tangle becoming less as they grow farther from the center until they are only loosely woven together at the surface forming the outer covering. The cells of all the hyphae are very short and are densely filled with a finely vacuolated cytoplasm. They are reg-

ularly binucleated, the nuclei showing the same structure as those found in the mycelium.

A section through a carpophore which is more mature shows the hyphae in the central portion arranged in parallel series vertical to the base. At the center there are a number of slender hyphae. Near the top these slender hyphae spread out and slightly downward to form the young pileus. The outer network of loose hyphae still encloses the growing cap and stipe. It is scarcely attached to the cap except near the apex where a few slender hyphae from the center of the pileus pass out into it. Below the cap it is continuous with the hyphae of the stipe. At this time previous to the formation of the gills the cells of the stipe have from four to eight nuclei. The cytoplasm is filled with large vacuoles.

Maire has described the formation and structure of the gills of *Clitocybe aurantiaca, Mycena galericulata, Stropharia semiglobata, Hypholoma appendiculata,* and *Psathyrella disseminata.* He finds that when the basidia are formed the cells of the trama are binucleated but in the stipe and pileus the cells are multinucleated. Harper has found this true also for *Coprinus ephemerus.*

I have also studied the young carpophore of other forms without growing them from spores to determine the arrangement of the nuclei in the young carpophore and in the mycelium in the substratum.

A species of *Crepidotus* was found growing on a rotten log in the green-house during the winter. The specimens were fixed with a portion of the substratum. For the most part the hyphae fill the vascular elements of the wood densely and are difficult to distinguish. But there are also single hyphae which grow in some of the large ducts that are favorable for study. The cells are very poor in protoplasmic contents. Two nuclei lie close together near the center of the cell surrounded by a little granular cytoplasm. At the surface the mycelial hyphae come together and twine around each other forming a short tangled mass which is the base of the carpophore. The hyphae branch profusely and curve upward growing in a vertical direction to form the

stipe. At the top of the stipe they spread out in every direction to form the pileus and gills. The cells are regularly binucleated and are filled with very dense cytoplasm.

*Certicium lilacino-fuscum* was also studied. It appeared in great abundance on a damp log in the green-house. Portions a quarter of an inch square were removed with a thick layer of the substratum and fixed in Flemming's weaker solution.

The surface layer is formed of densely packed short celled hyphae which grow perpendicularly to the substratum. The hyphae branch repeatedly, the branches crowding in between the hyphae. The cells are long and slender and so closely crowded together that I could make out the contents in only a few cases. In favorable preparations in a few instances cells were seen with two nuclei near the center and the remainder of the cell was filled with very granular cytoplasm. The clamp connections between adjacent cells are nearly always present. On the lower surface there are a large number of thick walled hyphae, destitute of cytoplasm, which form a loose felted layer and then penetrate the wood for some distance. They probably serve as a protective layer and also hold the mat close to the substratum. Among these basal thick walled hyphae there are others with thin walls and protoplasmic contents which also penetrate the wood in every direction and are usually single but occasionally are found in masses filling a vascular element of the wood. They are formed of long slender cells with two nuclei.

### *Rhizomorphs.*

In many *Agarics* the hyphae form mycelial strands which often grow to great length. These strands may branch frequently but without regularity and resemble roots. In some species the branches anastromose frequently forming a loose network; a character which is especially pronounced in *Dictyophora duplicata.* The structure of these so-called rhizomorphs is best known through the descriptions of *Armillaria mellea* by De Bary, Goffart and others. The forms which I have studied show a much simpler structure but they apparently have the same origin, the same general character of growth and doubtless serve the same general function. They provide a storage for reserve

material, a means of spreading the species in the substratum and are capable of retaining their vitality during a long dormant period.

*Poria.* (Fig. 19.)

The rhizomorphs of a species of *Poria* were collected in April from the underside of a board sidewalk where they had hibernated. Thus they were in a resting rather than the active growing condition of the other species described later. The main strands were about an eighth of an inch in diameter. These branch dichotomously a few times and from them a number of very slender branches are formed. These branches extend in every direction over the substratum; they subdivide freely, the branches frequently anastomosing and flattening out to form web-like expansions. The younger portions of the strand are a dull yellow while the older portions are coated with a yellowish brown layer.

Their structure is very simple. The entire strand is formed of slender hyphae which are very straight, only twining about each other slightly. At the center a large number of the hyphae have lost their contents and the walls are very thick. But among them are other hyphae with thin walls and rich protoplasmic contents. The cytoplasm is filled with small deeply staining bodies but there are no crystals. Towards the surface these thin walled hyphae become fewer and finally disappear leaving the outer layer formed wholly of thick walled hyphae (Fig. 19a). In a number of the thin walled cells two nuclei were found. They were very small and dense lying near together at the center of the cell. In some cases the deeply staining granules were so abundant and so closely packed that it was impossible to distinguish the nuclei. Among the hyphae there were numerous openings or pockets filled with octahedral crystals which dissolve slowly in hydrochloric acid indicating that they are calcium oxalate (Fig. 19c). Just beneath the surface layer of thick walled hyphae there is a layer of similar crystals very closely packed together (Fig. 19b).

*Pholiota praecox.* Pers. (Fig. 20.)

The mycelial strands of *Pholiota praecox* were collected from a mass of decaying leaves. A large number of mature caps were removed with all the connecting mycelial strands. The rotten leaves were easily pulled away leaving the strands free from all foreign matter. The strands were cut into pieces one *fourth* inch in length from the tip and from the older parts which show the branching.

The outside of the strand is covered by a thin layer of loosely tangled thick walled hyphae which doubtless serves as a protective layer. Beneath this is the main portion of the strand which has the same structure throughout with no differentiation into medulla and cortex. It is formed principally of large thin walled hyphae which show some slight regularity of arrangement although the longitudinal rows of cells so pronounced in Armillaria were not found. In some parts of the strands there are a few central hyphae that run very nearly straight. On both sides of this central strand the hyphae grow obliquely towards the surface. In other parts bundles of thirty or more hyphae, slightly entwined with one another coil around similar bundles or separate hyphae. Frequently it was impossible to make out any regular arrangement. The great majority of hyphae are formed of large cells but arising as secondary branches from them are a few narrow celled hyphae which run in the same direction as the larger hyphae until they reach the surface. At the surface instead of turning back towards the center as do the larger hyphae their walls become thickened and they form the felted layer. Thus the outer felt consists of the thickened terminal portions of the slender hyphae of the interior.

It is only by very careful staining with the triple stain that I was able to make out the contents of the cells. The walls of the large cells are partly gelatinized and have lost their smooth outline becoming wrinkled and creased. There is a very thin layer of cytoplasm with small light staining granules. The nuclei are flattened against the walls by the central vacuole. They are large and distinct with a very small nucleole on the side towards one end of the cell and a deeply staining body, probably a cen-

tral body, on the opposite side. The remainder of the nucleus is filled with a finely granular chromatin which takes a stain that is only slightly darker than that of the granules in the surrounding cytoplasm from which it is sharply separated by the nuclear membrane. All of the cells contain two and only two nuclei which may lie near together or separated by the entire length of the cell. Occasionally the cells had one or two small crystals.

The slender hyphae are formed of very long narrow cells which are filled with very dense cytoplasm. The nuclei are large, occupying nearly the entire diameter of the cell and lie close together at the center. When these hyphae reach the surface of the strand their walls become thickened leaving only a very small central strand of cytoplasm.

All of the cells have deeply staining convex plates on opposite sides of their cross walls. These convex plates always appear in pairs with their concave surfaces towards each other.

### *Lepiota naucina.* (Fig. 21.)

The mycelial strands of *Lepiota naucina* are very similar in appearance to those of *Pholiota*. They were found growing in the newly deposited mulch around the base of some young trees. It was impossible to obtain the mature carpophore attached to the strands but by loosening the substratum carefully I could obtain long strands still attached to the young buttons.

The structure of the strands differs in a few respects from those just described. In the interior of the strand the hyphae do not show any regularity of arrangement. The large celled hyphae are separated by a number of fine branches and are much fewer than in *Pholiota*. They are formed of large cells, with slightly rounded ends where the cross walls are formed. The slender hyphae arise as lateral branches of the larger ones and are very abundant. They wind among the larger hyphae forming a dense weft. Near the surface there is a layer of several cells thick entirely composed of slender hyphae. The cells are much shorter than in the center of the strand and each cell branches so that a close net-work is formed. Outside of this there is a felted layer of thick walled hyphae. The thick walled

hyphae were in this case so closely packed that it was impossible to trace their origin.

The contents of the cells are very similar to those of *Pholiota.* The large cells have a very thin peripheral layer of cytoplasm which contains a large number of small very deeply staining granules which may be very minute crystals. Larger crystals are very abundant, many of the cells containing twenty-five or thirty. They vary from the smallest size determinable to those which are one fourth of the diameter of the cell. They take a bright red color in the triple stain and are octahedrons in the majority of cases but a few appear to have more faces. Because of the numerous dark staining bodies, it was frequently impossible to differentiate the nuclei but in the cells where there were very few if any crystals or granules two nuclei were found. The large cells have wide pits in their walls through which it is easy to trace a protoplasmic strand. In a few cases the pore is so large that it is possible to trace a connection not only between the peripheral layers of cytoplasm but also between the central vacuoles. The protoplasmic granules frequently accumulate around these pores in large numbers but the convex plates described above are not formed. The slender hyphae are filled with a finely granular cytoplasm without any crystals or large granules. The cells are regularly binucleated. The convex plates are always present at their cross walls.

*Dictyophora duplicata.* Ed. Fisch. (Fig. 22).

The mycelial strands of *Dictyophora duplicata* were collected from the beds of mulch around the base of young trees on the University drive. By loosening the soil thoroughly large masses of the strands were obtained still in connection with young carpophores. The strands are fine and delicate with numerous irregular swellings. They branch frequently and the branches sometimes anastomose forming a very loose network.

The structure of the strands is decidedly different from the types already described. The very center of the strand is occupied by a few large hyphae which twine about each other slightly. They are formed of very long cells which have lost all of their contents and their walls are slightly gelatinized.

Towards the surface these large hyphae become much fewer and are separated by other hyphae which are only slightly smaller but very rich in protoplasmic contents. This second form of hyphae are the lateral branches of the first and correspond to the slender hyphae of the other species but are very different in appearance. They are loosely twined together towards the interior, forming an open mesh work through which a few of the larger hyphae run and also leaving numerous air-spaces. Towards the outside they become more and more closely woven together until they form a layer of densely packed hyphae just beneath the surface. Their cells are not so long as in the large hyphae but are nearly as wide in many places. They are irregular in width, especially towards the surface and contain large numbers of deeply staining bodies probably of a proteid character. These bodies take a deep blue color with the triple stain which requires a long exposure to Orange G. to remove. On account of the number of these dark bodies it was possible to locate the nuclei in only a few of the cells. In favorably stained cells there were two large nuclei with the usual structure, a small nucleole, and very finely granular chromatin, surrounded by a nuclear membrane with a deeply staining body at one side.

The hyphae do not have their walls thickened when they reach the surface as in all of the preceding species but their cells become shorter and very vesicular. The hyphae are very loosely woven together forming a very thick outer layer. The cells do not contain any of the dark bodies so abundant in the layer just beneath, but have a single large nucleus, which is very irregular in shape, near the center of the cell. This large nucleus is imbedded in a central mass of cytoplasm from which strands radiate in every direction towards the walls, as is the case in *Spirogyra* cells. I did not find any crystals in any of the cells or any convex plates at any of the cross walls.

*Lycoperdon pyriforme.* Schaeff. (Fig. 23.)

The mycelial strands of *Lycoperdon pyriforme* were found growing in a rotten stump. By breaking off pieces of the wood large quantities of the strands were obtained free from foreign

matter. The strands are fine and very tough, in marked contrast to the two preceding species.

In the center of the strand the hyphae run very nearly straight only slightly twining around each other. The large celled hyphae are formed of very long cells so that it was only occasionally that I found a cross wall. The walls are very delicate and do not show either a pore or the lens shaped plates. The cytoplasm forms a peripheral network of deeply staining strands which are very irregular and contain a number of large round granules. Only one or two octahedral crystals were found in any of the cells. The cells contain two large nuclei which take a very light stain as they have apparently only a small amount of chromatin. The nuclear membrane is very distinct and clearly defines the nucleus. The nucleole is very small.

The slender hyphae are very abundant and form the larger portion of the strand. They arise as lateral branches of the larger ones and twining among each other in a slightly oblique direction gradually work their way to the surface. At the surface they form a layer of densely packed hyphae by the interlacing of their branches. On the outside of this layer the hyphae are more loosely woven together and their walls become gradually thickened to form a protective covering. The cells of the fine hyphae are long and slender in the central portion of the strand, becoming much shorter as they approach the surface. At every cross wall very large distinct convex plates are present. The cytoplasm forms a finely granular layer at the periphery. There are always two large distinct nuclei near the center of each cell.

Section which pass through the main strand and the base of a lateral branch show that the hyphae which form the branch do not arise in any very definite fashion. Hyphae from various parts of the central and outer layers curve in the same direction towards one side and unite at the surface to form a branch. In one case I was able to trace a hypha which was growing on the other side of the strand, until it was nearly oposite the branch, where it curved sharply, crossed the main strand and entered the branch on the other side.

## CONCLUSIONS.

The results obtained from the above studies show that the binucleated cells do not originate through the formation of any special reproductive apparatus. Neither is there any structure in the formation of the hyphae or in the cells of the hyphae that indicates in any way where the binucleated cells will first appear. That they arise previous to the formation of a carpophore in a large number of forms is very evident. The long series of binucleated cells found in the mycelial strands or rhizomorphs of *Poria, Pholiota, Lepiota, Dictyophora* and *Lycoperdon* show that in these forms the origin of the binucleated cells is only distantly associated with the formation of a carpophore and it is probable that many strands never develop carpophores. In *Hypholoma perplexum* there is an expanded mass of mycelium with binucleated cells formed previous to the appearance of a carpophore. In many forms in which there is not any specialized structure between the filamentous mycelium and the carpophore, as in *Crepidotus, Corticium,* and in *Coprinus ephemerus* it was found that the cells of the mycelium in the substratum in the region of the carpophore were regularly binucleated. Binucleated cells were also observed in the cells of the mycelium of *Hypochnus* by Harper. As was mentioned previously, he was able to trace the series of binucleated cells from the hymenium into the mycelium in the substratum. The above cases show that the formation of binucleated cells is not necessarily followed immediately by the formation of a carpophore.

Further in the fairly complete series of stages from the germination of the spore to the mature carpophore which were obtained for *Hypholoma perplexum* and *Coprinus ephemerus* the same fact appears. A summary of the development of these species is as follows:

The spores of both species contain two sister nuclei which at germination pass from the spore into the germ tube. In *Coprinus* the nuclei divide very rapidly, forming a multinucleated germ tube which may contain twenty or thirty nuclei before the first wall is formed. The nuclei do not divide as rapidly in the germ tube of *Hypholoma* so that it seldom contains more than six or

eight when the first wall is formed. In the germ tube of *Hypholoma* the nuclei divide independently as was shown by the appearance of a single nucleus dividing in one of the germ tubes. A septate mycelium is formed in *Hypholoma,* the cells of which contain from one to nine or ten nuclei. The mycelial cells of *Coprinus* frequently contain as many as fifteen nuclei. Lateral branches are formed in both *Hypholoma* and *Coprinus,* the cells of which have a single nucleus. The cells are short and separate from each other readily. In *Hypholoma* these cells germinate forming a normal mycelium, thus proving conclusively that they are oidia. In *Coprinus* the cells did not germinate but their similar origin and structure indicate that they also are oidia.

Apparently after a varying period of growth hyphae are formed which have regularly two and only two nuclei in their cells. In the forms studied this association in pairs does not arise at any otherwise differentiated time or in any special structure.

We must remember as described above in *Coprinus* that binucleated cells were sometimes formed very near the spore, the main hyphae at a distance of two or three cells from the source of its origin having regular binucleated cells. In other cases special branches having binucleated cells were formed. When these branches arise from the uninucleated cells they probably receive a single nucleus which immediately divides. This division is not followed by a cross wall separating the two nuclei and thus the first binucleated cell is formed. If the branch having binucleated cells originates from a multinucleated cell it may receive two nuclei from the parent cell or it may receive a single nucleus that divides to form the second nucleus.

The hyphae with binucleated cells branch frequently and may develop immediately into a carpophore. This method of carpophore formation has been described for *Coprinus ephemerus.* The cells of the young carpophore are always binucleated and a series of binucleated cells extends to the formation of the hymenium. Thus the basidium with its two nuclei is the last of a long series of binucleated cells. That these two nuclei have maintained distinct lines of descent from the first cells with two nuclei occurring in the mycelium is not certain. I have not

found evidence of conjugate division in the hyphae but it seems possible that it exists and that the two nuclei of the young basidia have remained distinct throughout the series of binucleated cells.

The evidence seems satisfactory that in the rusts the series of nuclei in the binucleated cells are formed by the association of two nuclei in so called conjugate division. A wall is formed between the two pairs of daughter nuclei. Thus the two nuclei in a cell are not sister nuclei. The nuclei which fuse in the teleutospore have thus remained distinct through generations of binucleated cells. The wide occurrence of a series of binucleated cells in the young carpophore suggests a method of division similar to that in the rusts. Maire believes that the series of nuclei in the binucleated cells of the forms studied by him are formed by conjugate division but his figures do not seem conclusive. In *Hypholoma* and *Coprinus* each of the four spores borne on the basidium receives a single nucleus which immediately divides forming a binucleated spore. Maire states that a similar process occurs in *Clavaria vermicularis, Clitocybe aurantium, Mycena galericulata,* and *Amanita pantherina.* This resemblance between the nuclear history of the *Uredineae* and of the *Basidiomycetes* has been discussed fully by Maire, Harper and others.

In view of the fact that the hyphae with binucleated cells are found originating from uninucleated cells in *Coprinus,* it is very possible that further study may reveal some forms in which the mycelium will show regularly uninucleated cells and that this uninucleated series will extend to the first formation of a carpophore.

At present there is no evidence that the binucleated cells of the *Basidiomycetes* ever originate by a fusion of two adjacent cells such as Blackman (2) finds at the base of the aecidium in *Phragmidium violaceum* and *Gymnosporangium clavariaeforme.*

The occurrence of regular binucleated cells through a large part of the life history of the *Basidiomycetes* leading to the formation of basidia while binucleated cells are unknown in the life history of the *Ascomycetes* makes it difficult to consider the two groups as phylogenetically related. Maire (16) states that binucleated cells occur in the ascogenous hyphae in *Pustularia*

*vesiculosa, Galactinia succosa* and *Acetabula acetabulum.* But this hardly seems adequate proof for the conclusion he suggests. The origin of the ascocarp in a sexual process is in marked contrast to the origin of the carpophore as described above. It seems difficult to imagine that they are homologous structures. Certainly neither asci nor basidia can be considered as oogonia as Dangeard proposes.

That true cell fusion does not occur in *Coprinus* is very evident. It is doubtful how far this fact should influence our interpretation of the fusion of nuclei in the basidium. Two nuclei of more or less widely separated origin fuse and this is at least a common characteristic of sexual fertilization. Raciborski (19) believes that the binucleated cells of the rusts represent a prolonged vegetative stage intercalated between the cell fusion and the nuclear fusion just as in the zygospore of *Basidiobolus* a period may intervene between cell and nuclear fusion. He proposes the term zeugite for all cells in which occurs a fusion of nuclei belonging to the same cytoplasmic mass. But the fact that the binucleated cells of the *Basidiomycetes* are not the result of actual cell fusion makes it difficult to compare directly the delayed fusion in the zygospore of *Basidiobolus* and the nuclear fusion in the basidium.

Maire proposes to distinguish two types of fusion: "La sexualité avec fecondation" and "la sexualité avec mixie." The first type is found in the higher plants and animals, where the nucleus resulting from the fusion of two sexual nuclei contains twice the number of chromosomes contained in either of the fusing nuclei. The second type he believes is found in the lower plants and in the basidium. In this type the nucleus resulting from the fusion of two nuclei has the same number of chromosomes as was contained in each of the fusing nuclei.

Maire describes the nuclear fusion and division in the basidia in *Mycena galericulata, Psathyrella disseminata* and a large number of other *Basidiomycetes.* In all of these species he describes the two conjugate nuclei as each having two chromosomes and states that the fusion nucleus of the basidium in the first division has also only two chromosomes. But his figures are somewhat diagrammatic and decidedly inconclusive. On this evi-

dence Maire has formulated a theory of the alternation of generation in the *Basidiomycetes*. He believes that the origin of the binucleated cell is comparable to the fertilization of the higher plants. The carpophore having cells with paired nuclei corresponds to the sporophyte. The fusion of nuclei in the basidium is then not a fertilization but a reduction-process. Blackman's discovery that in the rusts the binucleated cells arise by an actual cell fusion is strong evidence in favor of this view and Blackman develops the conception into very satisfactory form so far as the rusts are concerned. It is possible that the origin of binucleated cells in the ordinary course of mycelial growth as described above may have come to serve as a substitute for cell fusions and that thus the stages of development with binucleated cells in the *Basidiomycetes* may be considered the equivalent of a sporophyte. Since, however, the point or points at which binucleated cells arise is very variable such a sporophyte can by no means be considered as representing a so definitely differentiated stage of development as does the sporophyte of the moss or fern.

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## PLATE IV.

## EXPLANATION OF PLATE IV.

All figures were drawn with the aid of the Abbé camera lucida.

Fig. 1. The spore of *Hypholoma perplexum* previous to germination. × 2700.

Fig. 2. Spore of *Hypholoma perplexum* with a short germ tube which has received a single nucleus from the spore. × 2700.

Fig. 3. Spore of *Hypholoma perplexum* with a short germ tube which has received both nuclei from the spore. × 2700.

Fig. 4. Same as Fig. 2.

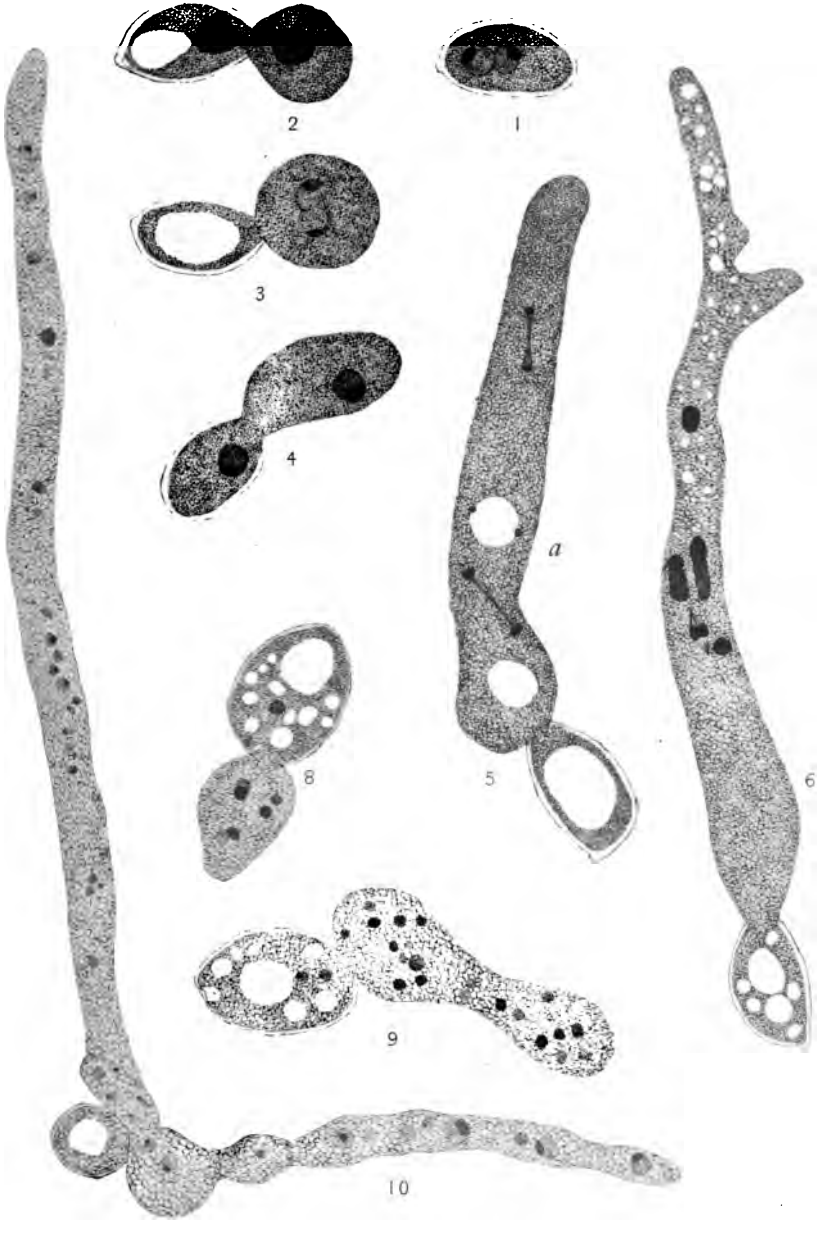
Fig. 5. The nuclei in germ tube of *Hypholoma perplexum* show late division figures. × 2700.

Fig. 6. One nucleus, in a germ tube of *Hypholoma perplexum*, is in the equatorial plate stage of division. × 2700.

Fig. 8. Spore of *Caprinus ephemerus* with a short germ tube containing several nuclei. × 2700.

Fig. 9. Same as Fig. 8.

Fig. 10. A germinating spore of *Caprinus ephemerus* at the end of sixteen hours. × 2700.

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## PLATE V.

## EXPLANATION OF PLATE V.

**Fig. 7.** The mycelium of *Hypholoma perplexum.* × 1200.

**Fig. 11.** Mycelium of *Caprinus ephemerus.* × 850.

**Fig. 12.** Oidial branches of *Caprinus ephemerus.* × 1500.

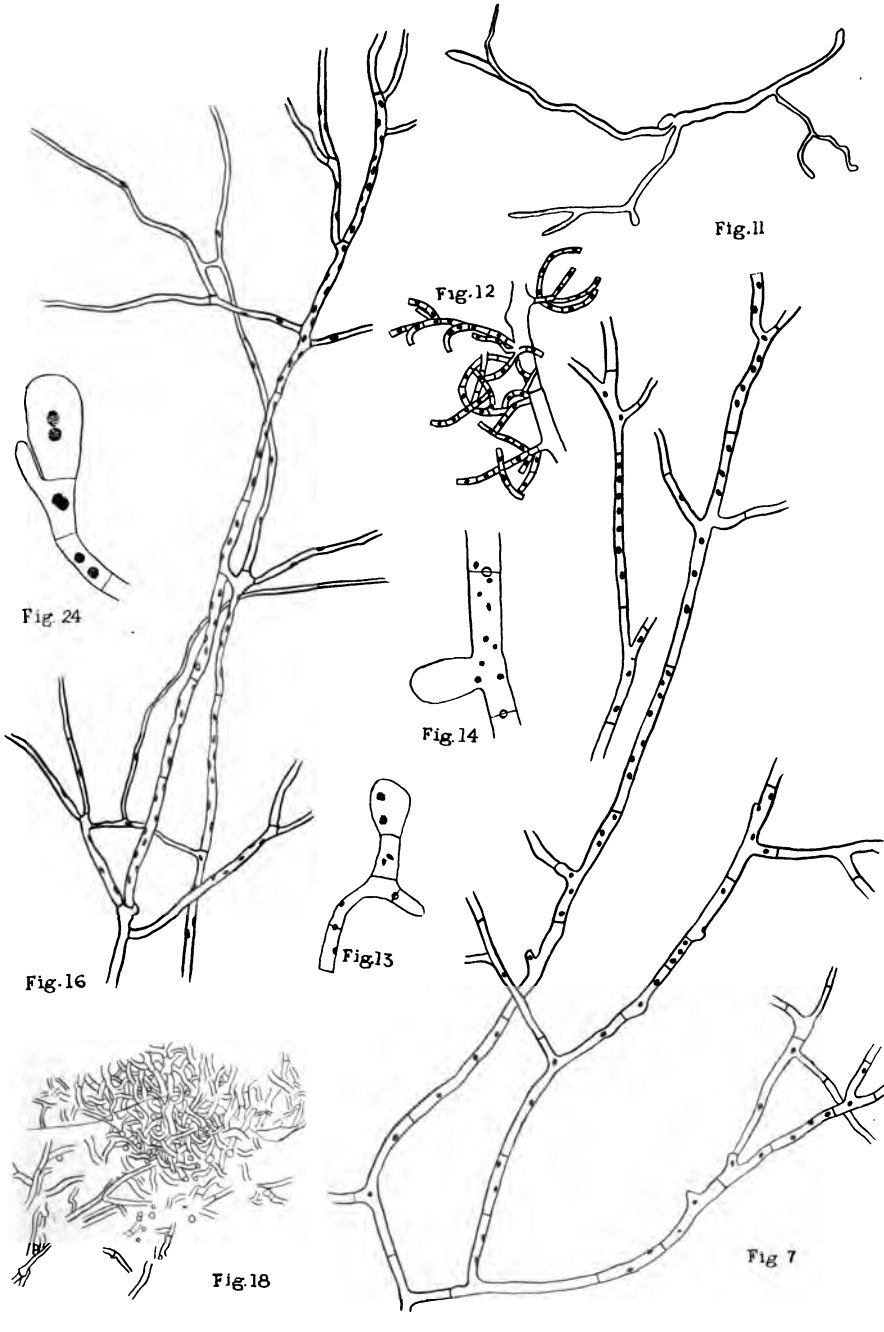
**Fig. 13.** A branch with binucleated cells formed on a hypha with uninucleated cells. × 1500.

**Fig. 14.** A large branch forming on a hypha with multinucleated cells × 1500.

**Fig. 16.** Mycelium of *Caprinus ephemerus* showing the arrangement of nuclei in the cells. × 1200.

**Fig. 18.** Section through the base of a young carpophore of *Coprinus ephemerus.* × 850.

**Fig. 24.** A section of *Hypholoma perplexum* showing the connection of a basidium with the subhymeniun. × 1500.

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## PLATE VI.

## EXPLANATION OF PLATE VI.

**Fig. 15.** The first indication of a fruit body of *Coprinus ephemerus*. × 1500.

**Fig. 17.** Same as Fig. 15. a, Y-shaped cell. × 1500.

**Fig. 19.** Longitudinal section of the rhizomorph of *Poria*. a, Outer layer of thick-walled cells; c, Pocket containing crystals; b, Layer of closely packed crystals. × 1200.

**Fig. 20.** Longitudinal section of the rhizomorph of *Pholiota praecox*. × 1200.

**Fig. 21.** Longitudinal section of the rhizomorph of *Lepiota naucina*. × 1200.

**Fig. 22.** Longitudinal section of the rhizomorph of *Dictyophora duplicata*. × 1200.

**Fig. 23.** Longitudinal section of the rhizomorph of *Lycoperdon pyriforme*. × 1200.

Fig.21

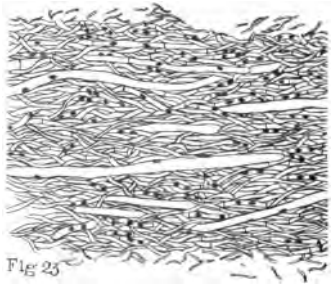

Fig 23

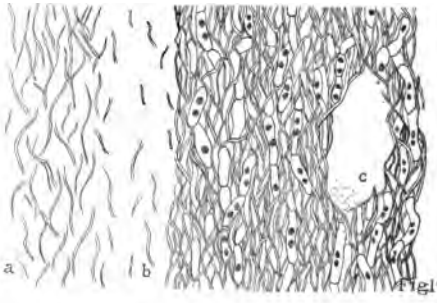


Fig19

Fig.22

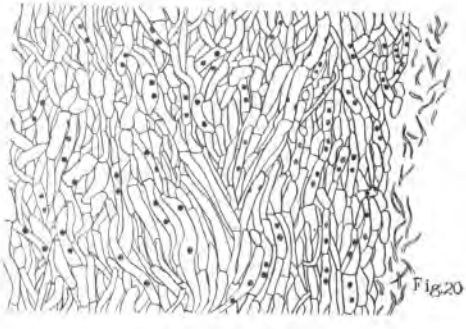

Fig.20

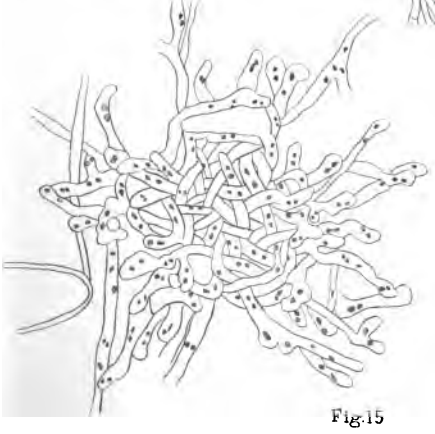

Fig.15

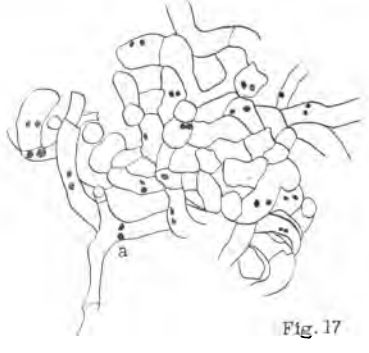


Fig. 17

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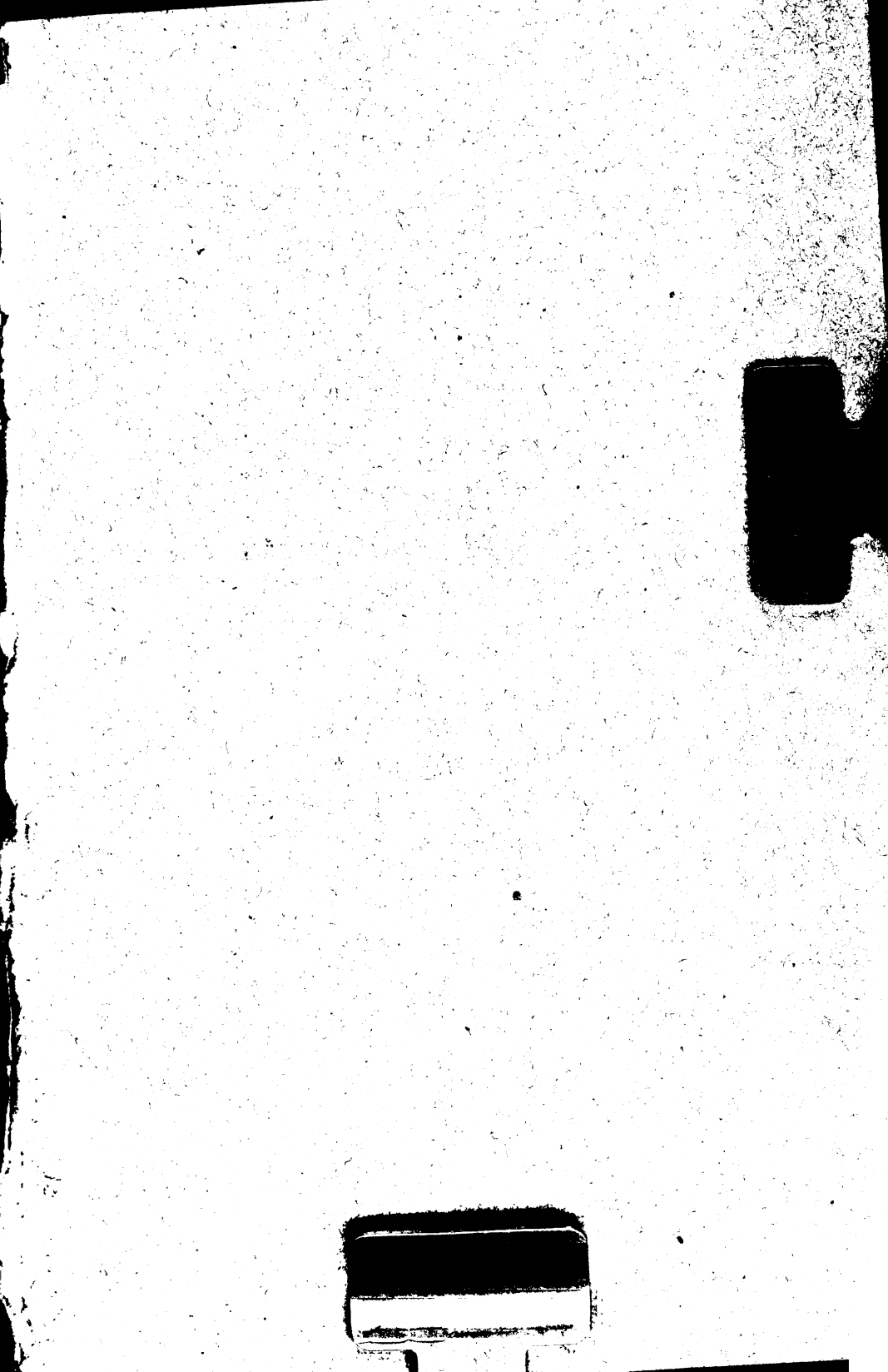

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